Target discovery for immunodiagnosis of invasive aspergillosis

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Antigens as targets for immunodiagnosis

- Principle
  - Microbial antigens are shed *in vivo*
  - Antigens can be detected by immunoassay
- Advantages of antigen detection diagnostics
  - Readily adapted to point-of-care format
  - If antigen is shed, may detect infection at peripheral sites
  - Proven technology; simple; inexpensive
  - Minimal sample preparation
- Examples
  - *Streptococcus pyogenes*
  - Rotavirus
  - RSV
  - Encapsulated bacteria
  - *E. coli 0157*
  - *C. difficile*
  - *H. pylori*
  - *C. neoformans*

Formats for antigen immunoassay

- Latex agglutination
- Antigen capture (sandwich) ELISA
- Lateral flow immunochromatographic assay
- Microbead-based immunoassay
- Microarray-based immunoassay

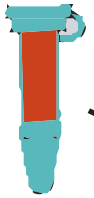
Targeting proteins for immunodiagnosis

- Many/most immunoassays target polysaccharides
- Advantages of proteins
  - Innumerable potential candidates
  - Usually very potent antigens – easy to raise antibodies
  - Can use bioinformatics for epitope prediction and assessment of “uniqueness”
- Disadvantages of proteins
  - Requires antibodies specific for two spatially independent epitopes – capture and indicator
  - Need for target discovery – which of the potentially hundreds or thousands of expressed proteins are shed into body fluids in amounts sufficient for detection by immunoassay?
- Question – Is it possible to take an unbiased look at target identification?

In vivo Microbial Antigen Discovery (InMAD)

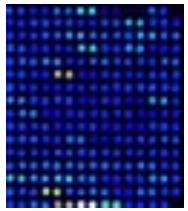
Infect BALB/c
A. fumigatus

Harvest serum & urine
when moribund
(InMAD serum)

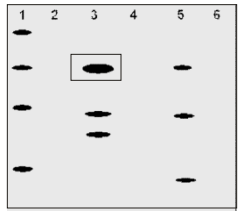


Immunize BALB/c
with serum or urine
from infected mouse

Polyclonal antibodies
(InMAD immune serum)



Proteome array



Immunoblot

Candidate antigen

InMAD identifies only those antigens that are shed in vivo

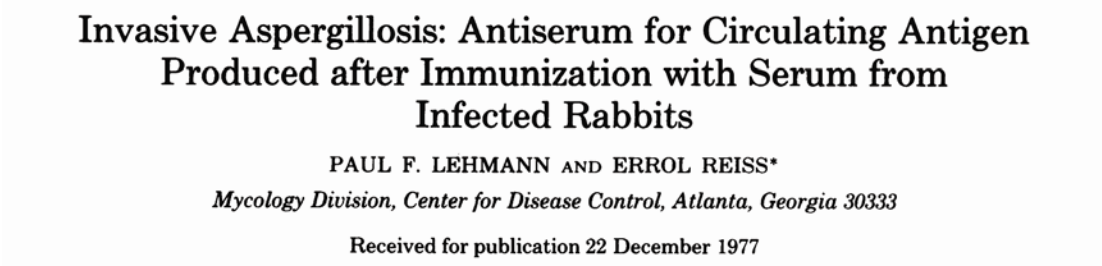
Additional strategies for target discovery

- Proteome arrays – probe arrays with InMAD immune serum
- Direct proteomic analysis
  - Collect serum or other body fluid from infected mouse or human
  - Identify microbial proteins
    - 1-D SDS-PAGE followed by LC-MS/MS
    - Multidimensional chromatography coupled to tandem mass spectrometry
- Once proteins are identified, assess uniqueness by query of databases

Target discovery for *Aspergillus fumigatus*

- Serum harvested from Aspergillus-infected BALB/c mice
- BALB/c mice immunized with serum from infected mice
- Immune serum collected
- Antigen prepared from *A. fumigatus*
  - Lysate
  - Culture filtrate
- Western blot probed with immune serum
  - Broad diffuse reactivity noted that is consistent with polysaccharide
  - Limited number of more discrete bands found that are consistent with proteins
  - Considerable mouse-to-mouse variability

Back to the future



Invasive Aspergillosis: Antiserum for Circulating Antigen Produced after Immunization with Serum from Infected Rabbits

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- Serum from an immunosuppressed and *A. fumigatus*-infected rabbit used to immunize a naïve rabbit
- Detected predominantly a single antigenic moiety in mycelial extract
- Detected antigen in serum and urine of infected rabbits
- Detected antigen in leukemic child with IA
- Subsequently identified as galactomannan

Summary

- Advantages of antigen immunoassay
  - Can identify antigens shed from distant site. e.g., IA
  - Proven technology
  - Easily adapted to point-of-care format
  - Can be very inexpensive
- Approaches to target discovery – varies with target category
  - Polysaccharides – candidates often known in advance
    - Capsules are obvious targets, e.g., *C. neoformans*
    - Other polysaccharides such as LPS or galactomannan
  - Proteins – rarely known in advance
    - Discovery complicated by very large number of potential targets
    - Target discovery by InMAD or alternative strategy such as proteomic analysis
    - InMAD has potential as a broad discovery platform